

# **EXHIBIT 1**

Sig

|  
PM-SM-BASE

wherein

PM is a phosphate moiety,

Enz-5(D8)(C2)

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is a phosphate moiety, SM is a monosaccharide moiety, and BASE is a pyrimidine, purine or 7-deazapurine moiety, said PM being attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE being attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group and such covalent attachment does not substantially interfere with double helix formation; and

(iii) a nucleotide having the formula

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine, and Sig is covalently attached to PM and such covalent attachment does not substantially interfere with double helix formation; and

(b) detecting the presence of said detectable Sig moieties in any of the oligo- or polynucleotides which have hybridized to said nucleic acid of interest.

285. The process according to claim 284, wherein Sig comprises at least three carbon atoms.

286. The process according to claim 284, wherein Sig comprises a monosaccharide, polysaccharide or an oligosaccharide.

287. The process according to claim 284, wherein Sig comprises a member selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

288. The process according to claim 287, wherein Sig comprises an electron dense component.

289. The process according to claim 288, wherein said electron dense component comprises ferritin.

290. The process according to claim 287, wherein Sig comprises a magnetic component.

291. The process according to claim 290, wherein said magnetic component comprises magnetic oxide or magnetic iron oxide.

292. The process according to claim 291, wherein said magnetic component comprises magnetic beads.

293. The process according to claim 284, wherein Sig comprises a sugar residue and the sugar residue is complexed with or attached to a sugar binding protein or a polysaccharide binding protein.

294. The process according to claim 293, wherein the binding protein comprises a lectin.
295. The process according to claim 294, wherein the lectin comprises Concanavalin A.
296. The process according to claim 294, wherein the lectin is conjugated to ferritin.
297. The process according to claim 287, wherein Sig comprises an enzyme.
298. The process according to claim 297, wherein the enzyme is selected from the group consisting of alkaline phosphatase, acid phosphatase,  $\beta$ -galactosidase, ribonuclease, glucose oxidase and peroxidase, or a combination thereof.
299. The process according to claim 287, wherein Sig comprises a hormone.
300. The process according to claim 287, wherein Sig comprises a radioactive isotope.
301. The process according to claim 287, wherein Sig comprises a metal-containing component.
302. The process according to claim 301, wherein said metal-containing component is catalytic.
303. The process according to claim 287, wherein Sig comprises a fluorescent component.
304. The process according to claim 303, wherein the fluorescent component is selected from the group consisting of fluorescein, rhodamine and dansyl.
305. The process according to claim 287, wherein Sig comprises a chemiluminescent component.

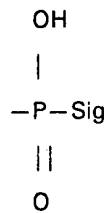
306. The process according to claim 287, wherein Sig comprises an antigenic or hapten component capable of complexing with an antibody specific to the component.

307. The process according to claim 284, wherein Sig is detectable when the oligo- or polynucleotide is contained in a double-stranded ribonucleic or deoxyribonucleic acid duplex.

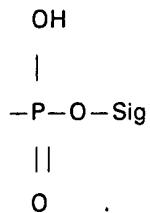
308. The process according to claims 284 or 307, wherein Sig is detectable when it is attached to the nucleotide directly or through a linkage group.

309. The process according to claim 308, wherein said linkage group does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

310. The process according to claim 284, wherein Sig in said nucleotide (iii) is covalently attached to PM via the chemical linkage



311. The process according to claim 310, wherein said chemical linkage comprises



312. The process according to claim 284, wherein the oligo-or polynucleotide is terminally ligated or attached to a polypeptide.

313. The process according to claim 284, further comprising contacting the sample with a polypeptide capable of forming a complex with Sig and a moiety which can be detected when the complex is formed.

314. The process according to claim 313, wherein the polypeptide comprises a polylysine.

315. The process according to claim 314, wherein the polypeptide comprises at least one member selected from the group consisting of avidin, streptavidin or anti-Sig immunoglobulin.

316. The process according to claim 313, wherein Sig comprises a ligand and the polypeptide comprises an antibody thereto.

317. The process according to claim 313, wherein the moiety which can be detected when the complex is formed is selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

318. The process according to claim 284, wherein the nucleic acid of interest is derived from a living organism.

319. The process according to claim 318, wherein the living organism is selected from the group consisting of prokaryotes and eukaryotes.

320. The process according to claim 284, wherein the sample is suspected of containing an etiological agent and the nucleic acid of interest is naturally associated with the etiological agent.

321. The process according to claim 320, wherein the sample is of human or animal origin and the etiological agent is selected from the group consisting of bacteria, virus and fungi.

322. The process according to claim 284, wherein the sample comprises a bacterium suspected of containing a nucleic acid of interest which imparts resistance to an antibiotic and wherein the oligo- or polynucleotide comprises a polynucleotide complementary to the sequence of the bacterium which confers resistance to the antibiotic.

323. The process according to claim 322, wherein when said bacterium is *Streptococcus pyogenes* or *Neisseria meningitidis*, said antibiotic is penicillin, wherein when said bacterium is *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, or *Neisseria gonorrhoea*, said antibiotic is a tetracycline, and wherein when said bacterium is *Mycobacterium tuberculosis*, said antibiotic is an aminoglycoside.

324. The process according to claim 284, wherein the sample is suspected of containing a nucleic acid of interest associated with a genetic disorder and wherein the oligo- or polynucleotide comprises a polynucleotide complementary to the nucleic acid associated with the genetic disorder.

325. The process according to claim 284, wherein the sample is suspected of containing a nucleic acid of interest associated with thalassemia and wherein the oligo- or polynucleotide comprises a polynucleotide complementary to the nucleic acid which is absent in the thalassemic subjects.

326. The process according to claim 284, wherein said process is utilized for chromosomal karyotyping which comprises contacting the sample with a series of the oligo- or polynucleotides which are complementary to a series of known genetic sequences located on chromosomes.

327. The process according to claim 284, wherein the sample is suspected of containing a nucleic acid which includes a terminal polynucleotide sequence poly A and wherein the oligo- or polynucleotide comprises a modified poly U molecule in which at least one uracil moiety has been modified by chemical addition of Sig to the 5' position of said uracil moiety.

328. The process according to claim 284, wherein said process is utilized to determine the number of copies of an individual chromosome in a sample.

329. A process for determining the sequence of a nucleic acid of interest, comprising the steps of:

incorporating one or more modified nucleotides into a nucleic acid or nucleic acid fragments complementary to said nucleic acid of interest, wherein said one or more modified nucleotides comprise a nucleotide modified on the sugar, phosphate or base moieties thereof, and wherein said one or more modified nucleotides are self-signalling or self-indicating or self-detecting, to produce a labeled nucleic acid or labeled nucleic acid fragments complementary to said nucleic acid of interest;

separating said labeled nucleic acid or labeled nucleic acid fragments in a sequencing gel; and

detecting the presence of each specific segment of said labeled nucleic acid or labeled nucleic acid fragments by means of said self-signalling or self-detecting modified nucleotide.

330. The process according to claim 329, wherein said incorporating step is carried out by means of one or more primers, nucleoside triphosphates or dideoxynucleotides.

331. The process according to claim 329, wherein said modified nucleotide comprises a member selected from the group consisting of:

(i) a nucleotide having the formula

PM—SM—BASE—Sig

wherein

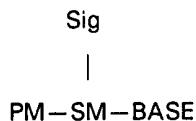
PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine 7-deazapurine, and

Sig is a detectable moiety,  
wherein PM is attached at the 3' or the 5' position of the monosaccharide  
moiety SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or  
5' position when said nucleotide is a ribonucleotide, BASE is attached to the  
1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup>  
position when BASE is a purine or a 7-deazapurine, and Sig is covalently  
attached to BASE at a position other than the C<sup>5</sup> position when BASE is a  
pyrimidine, at a position other than the C<sup>8</sup> position when BASE is a purine,  
and at a position other than the C<sup>7</sup> position when BASE is a 7-deazapurine  
and such covalent attachment does not substantially interfere with double  
helix formation;

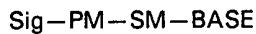
(ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,  
SM is a monosaccharide moiety,  
BASE is a pyrimidine, purine or 7-deazapurine, and  
Sig is a detectable moiety,  
said PM being attached to SM at a position independently selected from the  
2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and  
at a position independently selected from the 3' and 5' positions when said  
nucleotide is a deoxyribonucleotide, said BASE being attached to the 1'  
position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup>  
position when BASE is a purine or 7-deazapurine, and Sig is covalently  
attached SM directly or through a linkage group and such covalent  
attachment does not substantially interfere with double helix formation; and

(iii) a nucleotide having the formula



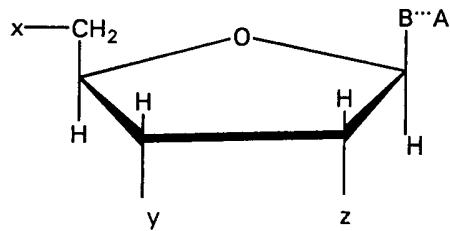
wherein

PM is a phosphate moiety,  
SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and  
Sig is detectable moiety,

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is purine, and Sig is covalently attached to PM and such covalent attachment does not substantially interfere with double helix formation.

332. The process according to claim 329, wherein said modified nucleotide has the structure:



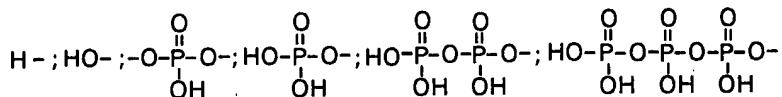
wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety suitable for incorporation into a polynucleotide and covalently bonded to the C<sup>1</sup>-position of the monosaccharide moiety, provided that when B is a purine or 7-deazapurine, the monosaccharide moiety is attached at the N<sup>9</sup> position of the purine or deazapurine, and when B is a pyrimidine, the monosaccharide moiety is attached at the N<sup>1</sup> position of the pyrimidine;

wherein A represents at least three carbon atoms and is an indicator molecule that is self-signaling or self-indicating or self-detecting selected;

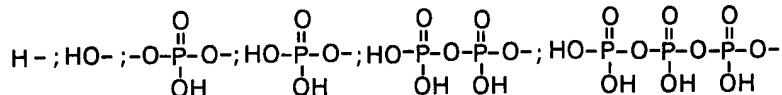
wherein B and A are covalently attached directly or through a linkage group, said linkage group not interfering substantially with detection of A;

wherein if B is a purine, A is attached to the 8-position of the purine, if B is a 7-deazapurine, A is attached to the 7-position of the deazapurine, and if B is a pyrimidine, A is attached to the 5-position of the pyrimidine; and

wherein x comprises a member selected from the group consisting of:



wherein y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of H- and HO-.

333. The process according to claim 329, wherein said self signalling or self-detecting modified nucleotide comprises a member selected from the group consisting of a fluorescent component, a chemiluminescent component, and a chelating component, or a combination of any of the foregoing.

334. The process according to claims 329 or 333, wherein said labeled nucleic acid or labeled nucleic acid fragments are detectable by a means selected from the group consisting of a fluorescent measurement and a chemiluminescent measurement, or a combination thereof.

335. The process according to claim 329, wherein the labeled complementary nucleic acid is fragmented prior to separation in said sequencing gel.

336. The process according to claim 329, wherein said incorporating step, the one or more modified nucleotides are incorporated in the presence of a primer.

337. A process for preparing a labeled oligo- or polynucleotide of interest, comprising the steps of:

(A) providing:

one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into an oligo- or polynucleotide, said chemical modification comprising a label capable of providing directly or indirectly a detectable signal indicating the presence of said labeled oligo- or polynucleotide, said chemically modified nucleotides being modified on the sugar, phosphate or base moieties thereof and being selected from the group consisting of:

(i)

PM—SM—BASE—Sig

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein PM is attached at the 3' or the 5' position of the monosaccharide moiety SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE directly or through a linkage group at a position other than the C<sup>5</sup> position when BASE is a pyrimidine, at a position other than the C<sup>8</sup> position when BASE is a purine, and at a position other than the C<sup>7</sup> position when BASE is a 7-deazapurine and such covalent attachment does not substantially interfere with double helix formation;

(ii)

Sig

|

PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein said PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group and such covalent attachment does not substantially interfere with double helix formation; and

(iii)

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety; and

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group and such covalent attachment does not substantially interfere with double helix formation; and

said oligo- or polynucleotide of interest; and

(B) incorporating said one or more modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest.

338. The process of claim 337, wherein said providing step Sig is covalently attached to BASE, SM or PM through a linkage group.

339. The process of claim 338, wherein said linkage group contains an amine.

340. The process of claim 339, wherein said amine comprises a primary amine.

341. The process of claim 338, wherein said linkage group does not substantially interfere with formation of the signaling moiety or detection of the detectable signal.

342. The process of claim 337, wherein said incorporating step is carried out using an enzyme.

343. The process of claim 342, wherein said enzyme comprises a polymerase.

344. The process of claim 343, wherein said polymerase comprises DNA polymerase.

345. The process of claim 337, wherein said one or more chemically modified nucleotides or said other modified or unmodified nucleic acids comprise a nucleoside di- or tri-phosphate.

346. The process of claim 337, wherein said incorporating step is template dependent or template independent.

347. The process of claim 346, wherein said incorporating step is template dependent.

348. A process for detecting the presence of an oligo- or polynucleotide of interest in a sequencing gel, comprising the steps of:

(A) providing:

(a) one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into an oligo- or polynucleotide, said chemical modification rendering said one or more chemically modified nucleotides either:

(I) self-signaling or self-indicating or self-detecting; or

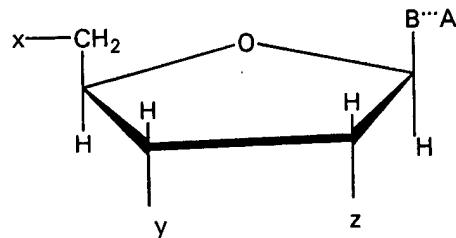
(II) comprising a label capable of providing directly or indirectly a detectable signal;

said self-signaling or self-indicating or self-detecting chemical modification or said label indicating the presence of said labeled oligo- or polynucleotide; thereby indicating the presence of said labeled oligo- or polynucleotide, said chemically modified nucleotides being modified non-disruptively or disruptively on at least one of the sugar, phosphate or base moieties thereof; and

(b) an oligo- or polynucleotide;

(B) incorporating said one or more chemically modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest, said labeled oligo- or polynucleotide of interest comprising one or more chemically modified nucleotides selected from the group consisting of:

(i)



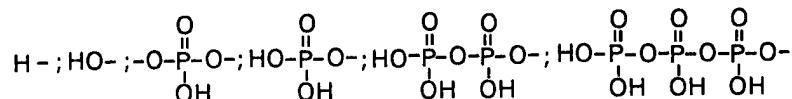
wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety covalently bonded to the C1'-position of the sugar moiety, provided that whenever B is a purine or 7-deazapurine, the sugar moiety is attached at the

N9-position of the purine or 7-deazapurine, and whenever B is a pyrimidine, the sugar moiety is attached at the N1-position of the pyrimidine;

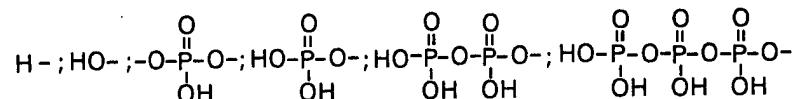
wherein A comprises at least three carbon atoms and represents at least one component of a signalling moiety capable of producing directly or indirectly a detectable signal or being self-signaling or self-indicating or self-detecting; and

wherein B and A are covalently attached directly or through a linkage group, and

wherein x comprises a member selected from the group consisting of:



wherein y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of H- and HO- ;

(ii)

Sig

|

PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein said PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a

pyrimidine or the N<sup>9</sup> position when BASE is a purine or 7-deazapurine, and  
Sig is covalently attached to SM directly or through a linkage group; and

(iii)

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety; and

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group;

(C) transferring said labeled oligo- or polynucleotide of interest to a sequencing gel;

(D) separating said labeled oligo- or polynucleotide of interest from other nucleic acids not of interest; and

(E) detecting directly or indirectly the presence of said labeled oligo- or polynucleotide.

349. The process of claim 348, wherein said incorporating step, A in the nucleotide (i) is covalently attached to B through a linkage group.

350. The process of claim 349, wherein said linkage group contains an amine.

351. The process of claim 350, wherein said amine comprises a primary amine.

352. The process of claim 348, wherein said incorporating step, Sig in the nucleotide (ii) is covalently attached to SM through a linkage group.

353. The process of claim 352, wherein said linkage group contains an amine.

354. The process of claim 353, wherein said amine comprises a primary amine.

355. The process of claim 348, wherein said incorporating step, Sig in the nucleotide (iii) is covalently attached to PM through a linkage group.

356. The process of claim 355, wherein said linkage group contains an amine.

357. The process of claim 356, wherein said amine comprises a primary amine.

358. The process of claims 349, 352 or 355, wherein said linkage group or groups do not substantially interfere with formation of the signaling moiety or detection of the detectable signal.

359. The process of claim 348, wherein said incorporating step is carried out using an enzyme.

360. The process of claim 359, wherein said enzyme comprises a polymerase.

361. The process of claim 360, wherein said polymerase comprises DNA polymerase.

362. The process of claim 348, wherein said one or more chemically modified nucleotides or said other modified or unmodified nucleic acids comprise a nucleoside di- or tri-phosphate.

363. The process of claim 348, wherein said incorporating step is template dependent or template independent.

364. The process of claim 363, wherein said incorporating step is template dependent.

365. The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide.

366. The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one external modified nucleotide.

367. The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one external modified nucleotide.

368. The process of claim 348, wherein said separating step is carried out electrophoretically.

369. The process of claim 349, wherein said detecting step is carried out directly.

370. The process of claim 348, wherein said direct detection is carried out on one or more self-signaling or self-indicating or self-detecting nucleotides.

371. The process of claim 370, wherein said one or more self-signaling or self-indicating or self-detecting nucleotides comprise fluoresceinated nucleotides.

372. The process of claim 371, wherein said fluoresceinated nucleotides comprise fluoresceinated DNA.

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